Testimony by John Vazzana before the
Senate Committee on Health, Education, Labor and Pensions

"Food Safety: Current Challenges and New Ideas
to Safeguard Consumers"

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Introduction

Intralytix, Inc is a biologics company focused on the development of bacteriophage-based products for the food safety, animal health, and human health markets. The emergence of antibiotic resistant bacteria has created a demand for new technologies to address health and safety problems existing in these markets.

Bacteriophages (phages) are a class of viruses that occur abundantly in nature and attack bacteria in a species-specific or strain-specific fashion. The use of phages in food safety and medical applications harnesses and makes more effective a widespread natural process that is already occurring in our environment, within our bodies, and on our food as we speak. The virtues of phage lie in their nearly unlimited ability to target existing and new bacterial pathogens, the complete safety of their use, and the ability to develop and deploy phage to counter new bacterial threats within a few months of detection.

Phages are the most-numerous life form on earth; some estimates place the phage population in the range of between 10^{31} and 10^{32}. In the environment, phages have evolved in parallel with their bacterial targets. They are robust entities that keep in check their bacterial-population counterparts and play an important role in the balance of all ecosystems.

Phages interact neither with humans, animals nor with plant cells, and therefore have a highly favorable safety profile. Phages have been used for several decades in Eastern Europe, and are effective in a number of situations where antibiotics are inadequate due either to bacterial resistance or poor blood supply; such situations include osteomyelitis, diabetic ulcers and severe burns.

"Simply stated, phages are viruses that infect bacteria. Like all viruses, phages are metabolically inert in their extracellular form and reproduce by insinuating themselves into the metabolism of the host bacteria. The viral DNA is then injected into the host cell, where it directs the production of progeny phages. These phages burst from the host cell, killing it and then infecting more bacteria. There are innumerable types of phages, each capable of eradicating its host bacterial species. They are abundant in the biosphere and can be produced on a large scale, very economically. It is important to note that phages only attack bacteria and have absolutely no adverse effect on humans, animals or the environment." *Company's website* *www.intralytic.com*

Phages were also used in the US and Europe during the early 20th Century. In the 1920s, Eli Lilly had at least seven phage products on the market. However, phages fell into disuse with the advent of broad-spectrum antibiotics. This was due to at least four reasons:

- Broad-spectrum antibiotics were easier to use than were phages, each of which have focused, narrow-spectrum activity.
- The medical crisis in wound treatment created by World War II, accelerating the demand for broad-spectrum antibiotics.
- Consistency, quality control and purity of phages (and phage therapy) were not always maintained.
- There was not broad consensus as to what phages were; two prevailing views had phages as either (1) viruses or (2) enzymes. For many, the actual nature of phages was settled only with the advent of electron microscopy, when the first images of phages (as virus particles) were finally obtained.

With the increasing threats from antibiotic resistant infections, phage research and development has increased sharply. Intralytix has developed products that address antibiotic resistant infections in wounds.

While phages were largely abandoned in the West, they continued to play an important role in the Soviet Union, where Giorgi Eliava established a research institute in Tbilisi, Georgia (Republic of Georgia) in collaboration with Felix d'Herelle, co-discoverer and prolific explorer of phages. That institute, now called the Eliava Institute, became the center for research and development of phage therapy.

Today, phage capabilities are still being developed in the former Soviet Union, particularly at the Eliava Institute in Georgia. Phages are also being explored by several U.S. and European firms, but no phages have yet to enter FDA-approved human trials. A couple of firms are pursuing veterinary or agricultural applications in the US and/or Canada. It is a principal objective of Intralytix to be the first company with phages in FDA-approved human trials.

Given the media attention to emergent infections and bioterrorism, it is not surprising that there has been significant mass-media coverage of phage therapy over the past couple years. Recent mention of the clinical potential of phages includes (but is not limited to):

1. Print media
    a. Science
    b. Wired – October 2003
    c. International Journal of Dermatology
    d. LA Times and NY Times
    e. Book: <u>The Killers Within</u> – has a chapter on phage therapy
    f. Recent story (9 December 2003) in the Star-Ledger newspaper in New Jersey
2. Other media
    a. Television programs
        i. Fox 5 Morning news

ii. CBS News
iii. BBC: The Virus that Cures
iv. 48 Hours
v. Canadian Discover program
vi. Dateline Australia
vii. The Nature of Things

b. A Canadian/French joint documentary film currently being made on phage therapy.

"Before penicillin became the medical world's darling, crusading doctors crisscrossed the globe armed with bacteriophages, bacteria killing viruses that, when administered to diseased patients via injection or potion, could be powerful healers" *U.S. News and World Report; Return of a killer -Phages may once again fight tough bacterial infections; November 2, 19*98

Intralytix

Intralytix was founded in 1998 by a group of business and technology leaders in Baltimore, Maryland. Today the founders make up the majority of the Board of Directors. A brief resume of each Board member is attached. The initial funding of the Company was provided by development partners interested in the development of products that would make their products safer. As a result of a development contract with Perdue Farms, the Company was able to develop products effective against *Listeria* and *Salmonella*. Agreements with Alpharma have resulted in the development of animal health products effective against *Salmonella* and *Clostridium perfringens*.

Founders

Dr. Torrey C. Brown, MD is the Chairman of the Board of Intralytix. Dr. Brown is the former State of Maryland Secretary of Natural Resources and is the former CEO and current chair of Family Health International. During Dr. Brown's tenure Family Health International grew from $9 M to $100M in annual revenues. He is a former Assistant Dean of the Johns Hopkins Medical School and member of the Maryland State Legislature, having served for twelve years.

Dr. J. Glenn Morris, Jr., MD is currently the Chairman of the Department of Epidemiology and Preventive Medicine at the University of Maryland Medical School, as well as Professor of Medicine and Professor of Microbiology and Immunology. He is an experienced infectious disease physician, epidemiologist, and specialist in food safety. From 1994-96 he was Director of the Epidemiology and Emergency Response Program at the Food Safety Inspection Service, USDA, and played a key role in the preparation of the 1995 USDA regulations on microbial safety in meat processing (the HACCP rule).

Dr. Sulakvelidze, a co-founder of Intralytix, received his formal training in microbiology in the former Soviet Union, including a B.A. from Tbilisi State University, a Ph.D. from Tbilisi State Medical University, and specialized training at the Engelhard Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia, and the University of Maryland School of Medicine, Baltimore, Maryland, USA.

Dr. Sulakvelidze's research interests are in the broad areas of emerging infectious diseases, molecular epidemiology, pathogenesis of diseases caused by bacterial enteric pathogens, bacterial toxins, and phage therapy. One of the major focuses in Dr. Sulakvelidze's research are studies of the potential usefulness of bacteriophages in preventing and treating infectious diseases caused by multidrug-resistant bacteria. The ability of lytic phages to reduce/eliminate colonization with, and treat diseases caused by, vancomycin-resistant enterococci, imipenem-resistant *Pseudomonas aeruginosa*, various *Salmonella* serotypes, and other bacterial pathogens have been studied. Dr. Sulakvelidze is also actively involved, in close collaboration with the Maryland Department of Health and Mental Hygiene, with studies of emerging infectious diseases. These studies include molecular epidemiological characterization of selected pathogenic strains by modern molecular typing techniques (PFGE, AP-PCR, etc.) and active participation in Maryland's Emerging Infectious Diseases Program (EIP) sponsored by the CDC.

Gary Pasternack, M.D., Ph.D., a co-founder of Intralytix, is a pathologist with extensive experience as a principal and a consultant in biotechnology businesses. Formerly he was the Director of the Division of Molecular Pathology at the Johns Hopkins University School of Medicine. Dr. Pasternack has served as member or chair of numerous review panels for the National Institutes of Health and the United States Army Medical Research and Materiel Command; he currently serves on a panel reviewing SBIR applications for the National Cancer Institute.

Patrick Hervy, a co-founder of Intralytix, is an experienced businessman who holds an MBA from Wharton. He is the founder of, Chairman and CEO of XLHealth Corporation. He is a member of the Board of Directors of Paragon Biotech, Inc. and has served as the former Chairman of MdBio, Inc. He is the former Chief Executive Officer of United States operations for Thomsen CGR.

John Woloszyn, JD, has been a business attorney with over 25 years experience representing technology-based companies. Mr. Woloszyn is a corporate attorney for multiple biotech, medical device, information technologies, and Internet companies. He has extensive experience in mergers, acquisitions, capital formation and the development of emerging growth companies. He is a member of the Board of Directors of MdBio, Inc., Chairman of the Board of Directors for Lombard Securities, Inc. and Chairman of Primaryimmune Services, Inc. He was a former Co-Vice Chair of Greater Baltimore Technology Council and member of the board of the NASA/Goodard Emerging Technologies Center in Baltimore, Maryland

Nina Siegler, CFA, a co-founder of Intralytix, is an expert in licensing and technology transfer. Ms. Siegler is a former Wall Street biotech analyst who later went on to found the technology transfer office at the National Institutes of Health. Ms. Siegler is the former head of technology transfer for the Johns Hopkins University at Homewood.

Existing Products

As a result of the strategic alliance with Perdue Farms, the company has developed products effective against *Listeria* and *Salmonella*. The products can be used as food safety and animal health products. The *Listeria* product, LMP 102, has been approved by FDA as a food additive.

The FDA approval gives us a template for future food additive products. We intend to submit a petition to FDA before the end of 2006 for an *E-coli* 0157:H7 food safety product. Thee product can be used on both red meat, and fruits and vegetables such as lettuce and spinach. Our proposed regulation will be identical to the regulation approved for LMP 102. We would hope this would help expedite the approval process.

Intralytix will submit a food additive petition to FDA in the second quarter of 2007 for prevention of *Salmonella* in poultry and eggs.

As a result of our research with *Salmonella*, we have developed a *Salmonella* vaccine that has proven to be very effective in reducing *Salmonella* colonization in poultry. When administered to newborn chickens, it reduces *Salmonella* colonization. In a study conducted by Perdue, Perdue reported that the Company's vaccine not only reduces colonization, but also improves the feed conversion ratio of the flock.

During the development of our *Salmonella* vaccine, we discovered that vaccines created using the company's phage-based technology appear to have better immunogenicity than vaccines created with standard technology. We believe this is an important technology platform for future products, initially in the field of animal health, but eventually for human health.

We currently have environmental products effective against *Salmonella* and *Listeria*. We have submitted our *Listeria* product to EPA for their approval. We believe the product has a market in food processing facilities.

PhagoBioDerm is a novel bandage-like wound-healing preparation consisting of a biodegradable polymer impregnated with antibiotic and bacteriophages that was recently licensed for sale in the Republic of Georgia (one of the former Soviet Union republics). PhagoBioDerm is the trade name for a 0.2-mm-thick, perforated wound dressing prepared as 4 x 5 cm films having a white/light yellow color. The films are impregnated with a mixture of lytic bacteriophages, an antibiotic, an analgesic, and sodium hydrocarbonate. The phage preparation is available commercially in the Republic of Georgia, and includes lytic bacteriophages active against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus,* and *Proteus*.

Technology

Bacteriophages, the natural predators of bacteria, were one of the first specific antibacterial therapies to become available. In the earlier part of this century, bacteriophage therapy was commonplace. Eli Lilly & Co. listed several phage products until the early 1940's. Because of

variability due to the then-incomplete understanding of phage biology, and because the immediate need of the medical community was for broad-spectrum antibacterials, bacteriophage therapy fell out of favor in the West. Eastern European and Soviet scientists, however, continued to develop bacteriophage technology alongside antibiotics, recognizing the inherent safety of bacteriophages and their complementarities to antibiotics.

Bacteriophages are viruses that infect bacteria but cannot infect human or animal cells. At approximately 1/75,000th of an inch, bacteriophages are much smaller than their bacterial foes. The structure of a bacteriophage is similar to a lunar lander, with a hollow head packed with bacteriophage genes, a tunnel-like tail, and long spindly legs. Once the phage lands upon its prey, the core of its tail creates a channel communicating with the interior of the bacterial cell. The bacteriophage uses the channel to inject its own genes inside the bacterial prey. Once injected, the phage genes commandeer the host machinery and force it to construct new phages, as many as 200 within three-quarters of an hour. Eventually, the overproduction of phages bursts and destroys the bacterium, sending the newly minted phages forth to infect more bacteria. Several key differences render animal cells impervious to phage: [1] the receptors, or chemical signals to which phage initially bind are found on bacterial surfaces but not the surfaces of animal cells; [2] phage are adapted to inject genes through the cell wall of bacteria, not the completely different membranes of animal cells; and [3] phage can take over the cellular machinery of bacteria, but not the completely different machinery of animal cells.

Resurgent interest in phage technology in the West is largely due to the emergence of antibiotic-resistant organisms. The lay press is filled with reports of so-called super bugs that are resistant to all known antibiotics, including those of last resort. In the US, numerous hospitalized patients die each year because there is no effective antibiotic to treat their vancomycin-resistant *Enterococci*, or methicillin-resistant *Staphylococci*. Yet these same strains are sensitive to bacteriophages.

Phage therapy has great appeal. Data from Eastern Europe and the former Soviet Union indicate that bacteriophages are not only effective, but they are safe as well. Bacteriophages trigger no allergic reaction in humans. In fact, phages are extremely common in the environment, are regularly consumed in foods, and are found as unintended contaminants in a variety of medications, including commercially available vaccines widely used in the US. For example, there may be as many as 200,000,000 phages per milliliter of unpolluted water. There are virtually no reports of complications, environmental or clinical, associated with the use of therapeutic phages. Bacteriophages thus appear to be safe for many applications including food processing and sanitation as well as for direct therapeutic applications in humans.

Commercial use of bacteriophages occurred in the West in the 1930's and early 1940's as previously mentioned. Phages were listed and sold as biological therapies by Eli Lilly, E.R. Squibb and Sons, and Swan-Myers (Abbot Laboratories). These products were used in mixed infections of the soft tissues, infected surgical wounds of the abdomen and pelvis, and in nonspecific genito-urinary infections. The Pasteur Institute in Paris prepared and used phages on a case-by-case basis. In the East, the Ministry of Health of the former Soviet Union routinely licensed active phage preparations for use in humans for treatment of wound, enteric, and respiratory infections.

Environmental effects are extremely unlikely since bacteriophages are ubiquitous. Commercial development involves selection of the appropriate naturally occurring phages that specifically, selectively, and efficiently kill the desired bacteria. No phages selected for use in food processing, sanitation, or therapy are capable of so-called lysogeny, where phages of undesirable classes insert into and alter bacterial DNA. Lytic phages, the type exclusively used by Intralytix, destroy their bacterial hosts without the possibility of transferring DNA. In order to ensure the phages used are lytic, Intralytix sequences all of our phages, and look for any undesirable genes. Since the bacteriophages cannot proliferate in the absence of their specific host, they disappear and become undetectable shortly after the last bacterium is killed. Bacteriophages thus represent a self-cleaning modality that fades away after doing its work.

Bacteriophages were discovered by Twort and D'Herelle in the early part of this century. Because of their remarkable antimicrobial activity, phages were utilized for treating human infections almost immediately after their discovery, and they continued to be used therapeutically in the pre-antibiotic era worldwide. D'Herelle's commercial laboratory in Paris produced at least five phage preparations against various bacterial infections. In the United States, a large U.S. pharmaceutical company produced seven phage products for human use in 1940s, including preparations targeted against staphylococci, streptococci, *E. coli*, and other bacterial pathogens. These preparations were used to treat various infections, including abscesses, suppurating wounds, vaginitis, acute and chronic infections of the upper respiratory tract, and mastoid infections. However, with the advent of antibiotics, interest in phage therapy waned in the United States and Western Europe. Antibiotics offered the broad bactericidal coverage necessary to treat infections prior to the establishment of a definitive diagnosis, whereas bacteriophages were exquisitely specific for individual bacterial strains or species. As a result, virtually no subsequent research was done on the potential therapeutic applications of phages in either humans or animals in the West. However, phages continued to be used therapeutically - together with, or instead of, antibiotics – in Eastern Europe and in the former Soviet Union. Several institutions in these countries were actively involved in therapeutic phage research and production, with activities centered at the Eliava Institute of Bacteriophage, Microbiology, and Virology of the Georgian Academy of Sciences, Tbilisi, Georgia,

Intralytix is a pioneer US company working on therapeutic bacteriophages. The company has made a significant progress in bringing phage technology to the cutting-edge biotech level by (a) identifying novel, commercially important applications for phage technology, (b) utilizing expertise from eastern European and former Soviet Union countries to adapt and improve state-of-the-art phage technology, (c) applying modern scientific approaches to better understand phage biology and phage-bacterial cell interactions, and (d) utilizing modern, state-of-the-art, biological processing technology. To this end, Intralytix has achieved a number of significant milestones, and it possesses significant expertise in the field that positions it well ahead of the competition. For example, Intralytix has

(i) optimized phage isolation and propagation techniques, which enabled the company to construct a large library of monophages against various multidrug-resistant bacterial pathogens,

(ii) developed pertinent animal models for evaluating phage safety and efficacy,

(iii) delineated optimal phage delivery routes and dosage levels for environmental decontamination and clinical applications,

(iv) optimized purification procedures for obtaining highly purified and concentrated phage preparations, and

(v) determined optimal conditions for freeze-drying phages, which result in water-dispensable, easily transportable, and stable viable phage preparations.

Phages are "natural products," that are ubiquitous in the environment. For example, 1 ml of non-polluted water contains approximately 200,000,000 phages. Because of this, the environment is an excellent source for lytic phages; majority of Intralytix's phages, for example, were isolated from the waters of Baltimore Inner Harbor or Chesapeake Bay. Technologically, initial isolation of phages is a relatively straightforward procedure, and is an exercise often included in advanced college microbiology course laboratories. However, only a small fraction of all isolated phages will prove to have utility as a therapeutic agent. Identification of phages having broad lytic activity against a specific pathogen is a complex process, involving repeated isolation, propagation, and characterization of phages over a period of time. As noted above, Intralytix has proprietary technology for efficient phage isolation, identification, characterization, propagation, and purification. The company has used this technology to develop an extensive library of monophages targeted against various specific pathogens. This technology (and the resultant phage library) is one of the key elements in the ability of the company to rapidly move forward with commercialization of phage products.

For production, phages are produced in fermenter lots by growing them on their host bacteria. Subsequent separation and purification of phages, and removal of adventitious material, involves *know how* technology proprietary to the company. At that point, as per an Intralytix-developed procedure, various phage preparations are constructed by mixing several separately grown and well-characterized lytic monophages, in order to (a) achieve the desired, broad target activity of the phage preparation, (b) ensure that the preparation has stable lytic properties, and (c) minimize the development of resistance against the preparation. Phages and phage preparations can be stored as concentrated liquid preparations (stable for at least 6 months), or can be freeze-dried (viable indefinitely long).

In studies conducted by Intralytix, the Company's phages were highly effective in decontamination of environmental surfaces and electronic equipment. In studies conducted in collaboration with investigators at the Agricultural Research Service, USDA, aerosolized phage preparations have also been highly effective in reducing pathogens on various fruits and vegetables by several logs (from 100 to over 1000 fold). Thus, phages are proven to be highly effective in these settings. However, appropriate technology for phage delivery and optimal application methodologies must be developed for phage treatment to be maximally effective. *Intralytix has developed such technology.*

LMP 102

Identity and Formulation

LMP 102 is a phage preparation consisting of a mixture of equal proportions of six individually purified phage, each of which is specifically effective against genetically diverse *Listeria monocytogenes* strain populations. It is possible to optimize the effectiveness of the preparation by customizing for differences in *L. monocytogenes* strains and serotypes that predominate in different geographic regions of the country or that may be associated with particular food type facilities. Six different phages will always be used to provide robustness.

Bacteriophages have been isolated from drinking water and from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese.

LMP-102 is all natural product that contains six bacteriophages isolated from the environment. The phages have not been altered or manipulated in any way. The preparation is specifically targeted against *L. monocytogenes* – one of the deadliest foodborne bacteria that kill approximately 25% of the people infected. The product does not otherwise alter the general composition of the foods, and it triggers no adverse organoleptic changes (i.e., it does not alter taste, odor or color of treated foods). The product has no effect on food shelf life (i.e., it does not extend the shelf life of treated foods).

The product is all natural, and no media of animal origin has been used during its preparation. In addition, no known, potentially allergenic substances (wheat, milk, soy, etc.) have been added to/mixed with the product.

The phage component of LMP-102™ is roughly estimated to be 0.1 ppm by weight and the remainder is phosphate-buffered saline containing up to 125 ppm residual organics from the growth medium and biomass.

The LMP-102™ article of commerce is a liquid made up of six monophages that individually have a lytic titer of 9.0 ± 0.5 $\log_{10}$ plaque-forming units (PFU) per ml.

LMP-102™ Proposed Use Levels

It is proposed that LMP-102™ be allowed for use as an antimicrobial processing aid in the production of ready-to-eat (RTE) meat and poultry products. LMP-102™ article of commerce is applied to the surface of the RTE food articles just prior to packaging. For most RTE food articles, this will

require application of LMP-102™ at a rate of approximately 1 ml per 500 cm^2 (~2 µl/cm^2) of RTE food article surface area.

Directions for Use

Dispensing

Automated dispensing equipment will be used in most applications of LMP-102™. The dispensing equipment will be microprocessor controlled and will provide for accurate delivery of the phage solution to the specific application points. Dispensing equipment and commercial product package will have an integral "lock and key" connection device to prevent inadvertent dispensing of improper compositions. Dispensing system and package design will provide for near-complete evacuation of commercial product package to prevent excess discharge of active material to environment and waste stream.

Dispensing system will have an integrated clean-in-place (CIP) system to provide daily, or as required, cleaning and sanitizing of the dispensing system.

Application

The application mechanics may be different for each type of RTE food article treated with LMP-102™ solution. In all applications, the phage solution will be spray applied onto the RTE food article surface. Low volume (low flow rate), low-pressure spray nozzles will be utilized to accurately dose the phages to all surfaces of the RTE food article. In some cases air-assisted spray nozzles may be employed to provide additional motive force to the low volume spray.

Description of Intended Technical Effect

LMP-102™ is intended to produce significant reduction of *L. monocytogenes* contamination vs. a water control when applied as directed to ready-to-eat (RTE) food products. LMP-102™ is further intended to produce significant reduction of *L. monocytogenes* contamination vs. an untreated control when applied as directed to RTE food products. In general, the reduction of *L. monocytogenes* contamination is better than 90% and often better than 99%"

Categories of Ready-to-Eat Food Products

LMP-102™ is intended to reduce *L. monocytogenes* contamination on a broad spectrum of RTE food products. RTE food products are products designed and labeled for consumption by the consumer without cooking at temperatures sufficient to kill any microbial contaminants that might be present. The following table represents categories of RTE meat and poultry products along with representative items in each category. The rationale behind the efficacy studies described in this section is that successful production of the intended technical effect on a foodstuff in a given category is indicative of efficacy among members of that category in general.

Categories of ready-to-eat food products

	Food category	**Example**
1	Cooked cured comminuted products, red meat	Beef frankfurters
2	Sliced cooked cured whole muscle cuts, red meat	Corned beef
3	Injected whole cooked muscle cuts, red meat	Flavored roast beef, uncured, water added
4	Sliced cooked whole muscle cuts, uninjected, red meat	Roast beef, minimally processed
5	Cooked cured comminuted products, poultry	Turkey frankfurters
6	Sliced cooked cured whole muscle cuts, poultry	Turkey pastrami
7	Injected whole cooked muscle cuts, poultry	Roast turkey skin, uncured
8	Sliced cooked whole muscle cuts, poultry	Roast turkey, minimally processed
9	Sliced cooked comminuted meat products	Sliced bologna, beef & pork
10	Sliced cooked comminuted poultry products	Sliced bologna, turkey
11	Uncured fermented comminuted red meat Products	Lebanon bologna
12	Uncured fermented comminuted poultry	Uncured turkey salami

Summary of Efficacy Data

Description of Test System

Efficacy studies were carried out under good laboratory practices (GLP). Twenty-seven samples of each of the 12 RTE products were inoculated on one surface with approximately 2 x 10^3 CFU per cm^2 of a 1:1:1 mixture of three *L. monocytogenes* strains, *L. monocytogenes* ATCC 19115 (serogroup 4b), *L. monocytogenes* Lm 68 (serogroup 1/2b), and *L. monocytogenes* Lm 82 (serogroup 1/2a). Samples were incubated for 20 ± 1 min at room temperature to allow for bacterial attachment. Nine samples of each inoculated RTE product were treated with LMP-102™. Nine samples of each inoculated RTE product were treated with a water control. The LMP-102™ and water control were applied to RTE product samples in a spray, using an airbrush adjusted to deliver 100 ± 20 µl per 4 seconds. All RTE product samples except frankfurters were sprayed for four seconds. Frankfurters were sprayed for a time period dependent upon their surface areas.

Following treatment, samples were vacuum packed and stored at 5 ± 2°C for 24 ± 4 h, 72 ± 4 h, or 168 ± 4 h. Samples were then analyzed for populations of *L. monocytogenes*. Phosphate buffered dilution water (PBDW, 100 ml) was added to the packages containing the RTE product samples, which were subsequently stomached. The resulting stomachates were serially diluted in PBDW and plated on MOX. Petri plates were incubated at 37 ± 2°C for 48 ± 4 h. The GLP Efficacy Study Report is included in Appendix F01.

Summary of Results

Compared with 250 ppm synthetic hard water only, LMP-102™ applied at a rate of approximately 1 ml per 500 cm^2 (~2 µl/cm^2), reduced populations of *L. monocytogenes* by 1.0-2.75 logs on all RTE products evaluated at 24 ± 4, 72 ± 4, and 168 ± 4 hours of storage at 5 ± 2°C. The reduction was statistically significant ($P < 0.05$). One exception was Lebanon bologna. Because Lebanon bologna exhibited intrinsic bactericidal activity against *L. monocytogenes*, recoverable populations in both treated and control samples were not obtainable in several instances, which resulted in a lack of variance in data. Thus, while application of LMP-102 appeared to reduce the levels of *L. monocytogenes* on Lebanon Bologna, statistical analysis was not possible in samples stored for 72 ± 4 and 168 ± 4 hours.

RTE product	Log_{10} reduction LMP-102™ treatment vs. water control		
	24 h	72 h	168 h
Beef frankfurters	1.91	1.45	1.25
Sliced ham	2.07	2.16	1.16
Flavored roast beef, uncured, water added	1.51	1.79	2.00
Roast beef, minimally processed	1.62	1.79	1.35
Turkey frankfurters	1.71	1.18	1.28
Turkey pastrami	1.48	1.88	1.83
Roast turkey skin, uncured	2.11	2.53	2.61
Roast turkey, minimally processed	1.49	1.36	1.33
Sliced bologna, beef & pork	2.34	2.69	2.45
Sliced bologna, turkey	2.67	2.57	2.75
Lebanon bologna	0.62	1.00	1.00
Uncured turkey salami	1.99	1.97	1.90

Safety of LMP-102™ Components

Safety of the Phages

Background exposure to phages and phage ubiquity

The safety and ubiquity of bacteriophages have been well established. The pertinent safety data on bacteriophages is briefly reviewed below. The published literature on phages, and other information developed by Intralytix, shows that:

- Bacteriophages are arguably the most ubiquitous organisms on earth. For example, one milliliter of non-polluted stream water has been reported Bergh et al., 1989 to contain approximately 2

x 10^8 PFU of phages/ml (Appendix H01), and the total number of phages on this planet has been estimated to be in the range of 10^{30}–10^{32}. This abundance of phages in the environment, and the continuous exposure of humans to them, explains the extremely good tolerance of the human organism to phages.

- Phages have been used therapeutically in humans for more than 80 years, without any recorded illness or death. During the long history of using phages as therapeutic agents in Eastern Europe and the former Soviet Union (and, before the antibiotic era, in the United States, France, Australia, and other countries), phages have been administered to humans (i) orally, in tablet or liquid formulations, (ii) rectally, (iii) locally (skin, eye, ear, nasal mucosa, etc.), in tampons, rinses and creams, (iv) as aerosols or intrapleural injections, and (v) intravenously, albeit to a lesser extent than (i) to (iv) – and there have been virtually no reports of serious complications associated with their use.

- Phages have also been administered to humans for non-therapeutic purposes without any recorded illness or death. To give just a few examples, phage preparations have been used extensively to monitor humoral immune function in humans in the United States in the 1970s-1990s, including in patients with Down's syndrome, the Wiskott-Aldrich syndrome and immunodeficient patients (Lopez et al., 1975; Ochs et al., 1982; Ochs et al., 1992; Ochs et al., 1993a;). In some of the studies (including FDA-performed studies), the purified phages were injected intravenously into HIV-infected patients or other immunodeficient individuals without any apparent side effects (Fogelman et al., 2000; Ochs et al., 1971; Ochs et al., 1993b).

- The biology of phages has been exhaustively studied. These studies have clearly shown that phages are obligate intracellular parasites of bacteria and are not infectious in humans or other mammals.

- Phages have been found in commercial sera and in FDA-approved vaccines commercially available in the United States (Merril et al., 1972; Milch and Fornosi, 1975; Moody et al., 1975).

- Bacteriophages are common commensals of the human gut, and they are likely to play an important role in regulating the diversity and population structure of various bacteria in human

GI tracts. Phages capable of infecting *E. coli*, *Bacteroides fragilis* and various *Salmonella* serotypes have been isolated from human fecal specimens in concentrations as high as 10^5 PFU/100 g of feces (Calci et al., 1998; Furuse et al., 1983; Armon et al., 1997). The recent data based on metagenomic analyses (using partial shotgun sequencing) of an uncultured viral community from human feces suggested that bacteriophages are the second most abundant category after bacteria in the uncultured fecal library (Breitbart et al., 2003).

- No adverse immunologic or allergic sequelae have ever been reported because of human or animal exposure to phages.